POTENTIAL APPLICATION DERIVED FROM THE ESSENTIAL OIL OF PANDAN LEAVES (*PANDANUS AMARYLLIFOLIS ROXB.)*

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ABSTRACT

Pandan (*Pandanus Amaryllifolius Roxb.)* is an erect green plant with the woody aerial roots and fan-shaped sprays of long, narrow, blade-like leaves leaf. It is widely used as natural flavoring in South East Asian dishes. Pandan leaves are also used by many people as an odour repellent in their cars due to its nature as an insect repellent and its aromatic fragrance. The major compound contributing to its aromatic fragrance is 2-acetyl-1-pyrroline (2AP). The potential usage of the essential oil as a natural air freshener was studied. The effects of the extraction temperature and period on quantity and quality of extraction yield were also studied. Experimental parameters included the temperature and extraction period. In this study, essential oil was extracted from pandan leaves by using the Microwave Assisted-solvent Extraction method, followed removal of ethanol solvent by using Rotary Evaporator. The chemical compounds inside extract included 2AP were identified by Fourier Transform Infrared Spectroscopy (FTIR), while the concentration of 2AP was analyzed by Gas Chromatography-Flame Ionization Detector (GC-FID). The concentration of 2AP for each parameter was determined by comparing the internal standard graph of trimehylpyridine (TMP) using GC-FID. The results shown that yield, volume of extracted essential and concentration of 2AP in essential oil increased with the extraction temperature before reaches the optimum point. Then, they decreased for higher temperature for extraction process due to decomposition of 2AP. On the other hand, the yield, volume of extracted essential oil and concentration of 2AP in essential oil decreased with increment of extraction period and then almost constant for longer period. This is because further exposure in high temperature for long period decomposed the heat sensitive compounds inside essential oil. Therefore, the extraction process at temperature 88^{o}C and 30 minutes produced the highest yield of extract and concentration of 2AP respectively. After the optimum condition of extraction method was determined, high amount of pandan leaves extract was produced for further research. Beaker A with 25 ml sample and beaker B with 25 ml water was given to 6 rooms in Kolej Kediaman 4 for sensory evaluation. The 6 rooms were chosen random, but have almost same environment condition. There are 5 people in each room to evaluate 5 criteria which are correctness, persistence, smell, intensity and overall acceptance for one week. They also measured the volume of water and sample left in beaker in every single day. Based on the result, most panels gave high rating grade for persistence, correctness and smell. However, they gave low rating grade for overall acceptance and intensity due to low concentration of 2AP in essential oil. The recommendation is to increase the concentration of 2AP inside essential oil in order to have high performance. The essential oil of pandan leaves has high potential to be a natural air freshener in daily life.

Keywords: Pandan leaves; extraction period; 2-acetyl-1-pyrroline; temperature; yield; volume; sensory evaluation; concentration 2AP; air freshener

ABSTRAK

Pandan (*Pandanus Amaryllifolius Roxb*.) merupakan tumbuhan hijau yang tegak dengan akar berkayu lembut dan daunnya berbentuk kipas lama, sempit, seperti pisau bilah. Ia banyak digunakan sebagai perisa semulajadi dalam masakan Asia Tenggara. Daun pandan juga digunakan oleh orang ramai sebagai bau penghalau dalam kereta mereka kerana harumannya and sifatnya sebagai penghalau serangga. Sebatian utama yang menyumbang kepada haruman aromatik 2-asetil-1-pyrroline (2AP). Potensi penggunaan minyak pati sebagai penyegar udara semula jadi telah dikaji. Kesan suhu dan tempoh pengekstrakan pada kuantiti dan kualiti hasil perahan juga dikaji. Parameter uji kaji termasuklah suhu dan tempoh pengekstrakan. Dalam kajian ini, minyak pati yang diekstrak daripada daun pandan dengan menggunakan kaedah pengekstrakan Microwave, diikuti penyingkiran pelarut etanol dengan menggunakan Rotary Evaporator. Bahan kimia di dalam minyak pati termasuk 2AP telah dikenal pasti oleh Fourier Transform Infrared Spectroscopy (FTIR), manakala kepekatan 2AP dianalisis oleh Gas Chromatography-Flame Ionization Detector (GC-FID). Kepekatan 2AP bagi setiap parameter adalah ditentukan dengan membandingkan graf trimehylpyridine (TMP) menggunakan GC-FID. Keputusan menunjukkan bahawa hasil, jumlah minyak pati dan kepekatan 2AP dalam minyak pati meningkat dengan suhu pengekstrakan sebelum mencapai titik optimum. Kemudian, mereka menurun dalam suhu yang lebih tinggi untuk proses pengekstrakan disebabkan oleh penguraian 2AP. Sebaliknya, hasil, jumlah minyak pati yang diekstrak dan kepekatan 2AP dalam minyak pati yang menurun dengan kenaikan tempoh pengekstrakan dan kemudian hampir malar bagi tempoh yang lebih lama. Ini adalah kerana pendedahan pada suhu yang tinggi bagi tempoh masa yang panjang terurai sebatian yang mempunyai sifat sensitif akan haba di dalam minyak pati. Oleh itu, proses perahan pada suhu 88^{o}C dan 30 minit menghasilkan quantity minyak pati yang banyak dan kepekatan 2AP tinggi masing-masing. Selepas keadaan optimum untuk kaedah pengekstrakan ditentukan, bnayak minyak pati diekstrak daripada daun pandan dihasilkan untuk penyelidikan selanjutnya. Bikar A dengan 25 ml sampel dan bikar B dengan 25 ml air telah diberikan kepada 6 bilik di Kolej Kediaman 4 untuk penilaian deria. 6 bilik yang dipilih secara rawak, tetapi mempunyai keadaan persekitaran yang hampir sama. Terdapat 5 orang dalam setiap bilik untuk menilai 5 kriteria termasuk ketahanan, kebetulan, bau, intensiti dan penerimaan keseluruhan selama satu minggu. Mereka juga mengukur isi padu air dan sampel yang tinggal dalam bikar dalam setiap hari. Berdasarkan keputusan itu, kebanyakan panel memberi gred penarafan tinggi untuk kebetulan, ketahanan, dan bau. Walau bagaimanapun, mereka memberi gred penarafan rendah untuk penerimaan keseluruhan dan intensiti yang disebabkan oleh kepekatan rendah 2AP dalam minyak pati. Dengan meningkatkan kepekatan 2AP di dalam minyak pati, minyak pati akan mempunyai prestasi yang tinggi. Minyak pati daripada daun pandan mempunyai potensi yang tinggi untuk menjadi penyegar udara semula jadi dalam kehidupan seharian.

Kata kunci: Daun pandan; tempoh pengekstrakan; 2-asetil-1-pyrroline; suhu; isipadu; hasilan; penilaian deria; kepekatan 2AP; udara penyegar.

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LIST OF SYMBOLS

^{o}C	Degree Celcius
ε̇	Dielectric constant
ppm	Parts per million
ppb	Parts per billion
W	Walt

LIST OF ABBREVIATIONS

2AP	2-acetyl-1-pyrroline
FTIR	Fourier Transform Infrared Spectroscopy
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GC-SPME	Gas Chromatography- Solid Phase Micro Extraction
MAE	Microwave-assisted extraction
RBD	Refined, bleached and deodorized
TMP	2,4,6-trimethylpyridine
UV-Vis	Ultraviolet-visible Spectroscopy

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CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 RESEARCH BACKGROUND

Pandanus amaryllifolius leaves, generally known as pandan, is a tropical plant which belongs to the screw pine genus (Pandanaceae). Pandan is an erect green plant with the woody aerial roots and fan-shaped sprays of long, narrow, blade-like leaves (Yahya et al., 2010). The leaves of pandan are dark green in colour with a strong nutty aroma. Rutuju Jathar (2011) stated that pandan plant is native to Asia and the tropical parts of Australia. It is a sterile plant and is often propagated by people by cutting the leaves.

The pandan leaves are widely used as a natural flavoring in South-east Asia including India, Thailand, Indonesia and Malaysia. For example, people usually cook non-aromatic rice with pandan leaves to impart a resemblance of leaf aroma which is similar to aromatic rice such as basmati and jasmine rice (Bhattacharjee et al., 2004). In addition, pandan leaves are sometimes added to iced drinks prepared from fresh unripe coconuts. Many people also extract the juice from pandan leaves and use it as essence in

cake making. Mohd Nor et al. (2007) stated that it gives a fragrant flavor to the savory dishes such as jellies, puddings, custards, chicken and sweets.

Besides having aromatic properties, pandan leaves are very beneficial for various health conditions. Whole pandan plant is considered to be diuretic because it contains traces of tannin, glycosides and alkaloids (Rutuju Jathar, 2011). Therefore, it is useful for healing various wounds and diseases such as smallpox. Apart from that, pandan leaves are usually used in aromatic therapy to relieve the weak nerves, so they are considered as the pain reliever to cure chest pain, headache, reduce fever, arthritis, earache and others. Moreover, pandan leaves are used by some parents as a healthy laxative for their children. Pandan leaves are also very effective in helping women with weak body recover after give birth. Some scientists found that the pandan plant is one of the anti-carcinogenic plants and is significantly useful for curing diabetes diseases. Several types of skin disorders including leprosy can be cured by pandan leaves too.

The essential oil derived from the pandan leaves contains a number of volatile compounds from groups of alcohols, aromatics, carboxylic acids, ketones, aldehydes, esters, hydrocarbons, furans, furnanones and terpenoids. In general, these volatile compounds are ethyl formate, 3-hexanol, 4-methylpentanol, 3-hexanone, 2-hexanone, trans-2-heptenal, β-damascenone, 4–ethylguaiacol and 3-methyl-2-(5H)-furanone (Yahya et al., 2010). Since the amounts of those compounds are very minimal, this study will focus on the compound 2-acetyl-1-pyrroline (2AP). It has a popcorn odor and an appearance from colourless to yellow. It has quite a high boiling point which is 182 ^{o}C, however it is a volatile component in the natural plant. The chemical formula of the compound 2AP is C_6H_9NO. Its molecular weight is 111.142 g/mol.

The major compound contributing to the aromatic flavor of pandan is 2-acetyl-1-pyrroline (2AP). Yahya et al. (2010) reported that 2AP is a hydrophilic compound which has the odor threshold value as low as 0.1 ppb in water. Besides, 2AP is a substituted pyrroline and a cyclic imine. In a study by Buttery et al., (1983), it is

reported that the quantities of 2AP present in pandan leaves (in unit of ppm) is 10 times and 100 times more than that found in the scented milled rice and common rice respectively. Therefore, pandan leaves are one of the best sources of 2AP compound. Li, and Ho. (2003) proved that seven compounds and fractions prepared from pandan leaves have repellence against certain species of cockroaches. From the study, it is proven that it has the potential to be a natural and environmental friendly pest management tool.

Several extraction methods are used by many researchers in the past for extraction of 2AP such as simultaneous steam distillation–solvent extraction (Buttery et al. 1986; Lin et al. 1990; Mahatheeranont et al. 2001), micro steam distillation–solvent extraction (Tanchotikul and Hsieh 1991) and direct solvent extraction (Mahatheeranontet al. 2001; Bergman et al. 2000; Yoshihashi 2002) coupled with analytical methods for quantification of 2AP.

1.2 PROBLEM STATEMENT

Pandan leaves are very popular among people of South East Asia as an aromatic flavoring in their daily activities. Sometimes, people will keep pandan leaves in refrigerator for 1 day before using them for cooking. However, the storage period of pandan leaves is very short, around 2-3 days. Pandan leaves will lose its fragrance slowly and change from green color to brownish color. Lastly, people have to replace the withered leaves with fresh pandan leaves. In addition, pandan leaves are harvested in a form of bunch with their roots intact. Some farmers bring these leaves to market and sell them to people in village. Since pandan have slender leaves which is proportional to its volume and weight, therefore it is unlikely to be carried around.

Synthetic air fresheners used in cars or living spaces will increase the risk of developing a variety of health problems. For example, Natural Resources Defense Council, (2007) reported that 12 of 14 brands of common household air fresheners contain phthalates. These phthalates are hazardous chemical compounds. They are often

used to prolong the length of time the scented products retain their fragrance, however they increase the risk of experiencing endocrine, reproductive, and developmental problems. Apart of that, some artificial air fresheners contain formaldehyde which is classified as a human carcinogen by the International Agency for Research on Cancer.

According to journal of Mathure (2010), there are many factors affecting the quality and quantity of 2AP extracted with every method. Those factors are pre-treatment methods, extraction time, extraction temperature, solvent quantity, sample weight and others. It is found out that the yield of 2AP from the HS-SPME extraction methods decreases when the extraction time is too long and temperature is too high. Therefore, it shows a need for optimization of those factors before proceeding. With the optimum condition for the experiment, a large amount of high quality 2AP can be obtained in the essential oil.

1.3 OBJECTIVES

The objectives of this research are:

i. To determine the optimum extraction time and temperature in order to get highest extraction yield of essential oil and concentration of 2AP.
ii. To develop a natural air freshener derived from the essential oil of pandan leaves

1.4 SCOPE OF RESEARCH

The research scope is very important to make sure that the objectives of research are achieved. Generally, the scope is used as a guideline for conducting this research. The scope of this research can be summarized as follow:

i. In this study, the type of raw material was restricted to *Pandanus Amaryllifolius Roxb.*. The pandan leaves must be fresh and purchased from the same place.
ii. The temperature of extraction process was set at 70^{o}C, 80^{o}C, 90^{o}C, 100^{o}C and 110^{o}C.
iii. The period of microwave assisted-solvent extraction process was set at 30 minutes, 40 minutes, 50 minutes, 1 hour and 70 minutes.

iv. The powers, ratio of raw materials to solvent, as well as pressure for the microwave assisted-solvent extraction of cut pandan leaves were consistent for all experiment runs.

v. The persistence period of essential oil was tested in rooms of Kolej Kediaman 4 in University Pahang.

vi. 30 people are chosen randomly for the sensory evaluation.

vii. The sensory testing period was one week.

1.5 RATIONAL AND SIGNIFICANT

Currently, many synthetic air fresheners are proven to pose risk of developing of a variety of health problems such as asthma, breathing problem headache and Bronchial irritation. Liu et al. analysed air freshener and reported that smoke from heating contains heavy metals, allethrin and hazardous phenol O-cresol. Sharma (2001) stated that the chemical, pyrethrums in air freshener could lead to running nose and wheezing, prolonged use will damage the liver corneal and cause asthma. This study aims to develop a natural air freshener and repellent from essential oil of pandan leaves to substitute those artificial products. Some chemical compounds contained in the essential oil are used in pharmaceutical. Therefore, the essential oil form pandan leaves will help to improve the human health through the aromatherapy while reducing the risk of getting health problems (Ruratech Services, 2010). Besides, the pandan air freshener is an environmental friendly product. It only contains non-hazardous chemical compounds meaning it is safer for use in daily life. The usage of pandan leaves will also minimize the amount of hazardous chemicals in the waste water resulted from the production of air freshener.

Since essential oil is kept in small bottles, it saves space and is more convenient to carry around compared to pandan leaves. It also has a much longer persistence period which makes it more economical as the pandan leaves will wither in a few days. Besides, the aromatic properties are suitable in eliminating unpleasant odors. Furthermore, the pandan plants can be grown easily in the South-East Asia since it can adapt easily to various environments and has a short growth period. With the abundance

of pandan leaves sources, pandan leaves have great potential for commercialization in future.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Pandan Leaves

Pandanus amaryllifolius Roxb. plant is known as pandan leaf. It is a tropical plant originally from Vietnam. *Pandanus amaryllifolius* Roxb.is grouped in the screwpine genus belonging to family *Pandanaceae* which is a paleotropic family of 800 trees and shrubs. Among the 36 species of this family that have been found in the India, *Pandanus odoratissimus* Linn. and *Pandanus amaryllifolius* Roxb. are of commercial interest to the food industry. This plant is seldom found in the wild but it is widely cultivated by people in South-East Asia. Nowadays, it is distributed over Southern India, peninsular South East Asia, Indonesia and Western New Guinea. It is an upright green plant and has woody aerial roots. It also has the fan-shaped sprays of long, narrow, bladelike leaves. Katzer, G., (2001) reported that this plant seldom has flowers and the flowers are not big bloomer. The male flowers in this species of plant are extremely rare, and there is no scientific evidence to prove the existence of the female flower. Katzer, G., (2001) also reported that the flowering pandan plants on the Moluccas archipelago are the only known instances. This is because this species have faced the evolution there by the process of hybridisation which shares its chromosome number ($2n$=60) with most other representatives of the genus.

It usually planted by people in container or ground. It can grow well in any kind of soil. Besides, it needs a lot of water and humid environment. It prefers to semi-expose to strong sunlight on afternoon. In general, it propagates by replanting the suckers which are formed at base or plantlets with aerial roots. However, the grounded suckers are better than aerial plantlets because some plantlets may fail to propagate. It can grow till a height between 6 feet and 8 feet tall. It also has leaves around 30-50cm long when it is mature. The pandan leaves exhibit the popcorn like, pleasant aroma. This aroma is special and hard to describe but a similar scent is found in some aromatic rice varieties grown in South East Asia. For examples, Buttery, et al., (1983) reported that the Thailand jasmine rice and Basmati rice have similar aroma but less dense. However, the pandanus leaves have strong scent only during wilting. The fresh, intact pandan leaves hardly have any odour. In addition, dried pandan leaves will lost its fragrance rapidly.

2.2 APPLICATION OF PANDAN LEAVES

In general, pandanus leaves are very popular natural food flavoring in tropical Asia, from South India to New Guinea. The food which use them as flavoring such as bakery products, sweets, ice-cream, yogurt, tea drink, and coconut jam. They also are used for others purposes, but mostly related to rice (Buttery et al., 1985). For example, in India and Philipines, pandan leaves are traditionally used during cooking the non-aromatic rice in order to impart the unique fragrance (Bhattacharjee et al., 2003). Therefore, people no need to buy the expensive aromatic rice. In Sri Lanka, they are widely used together with curry leaves to cook Singhalese curries. Moreover, in Thailand, Malaysia and Indonesia, some plain rice cooked in coconut milk together with pandan leaves is a delicacy even when eaten alone (Yahya et al., 2010). The Indonesian specialty *nasi kuning* and *nasi-lemak* are made by putting entire pandan leaf with coconut water and rice. Most delicious is rice steamed in small baskets made from pandanus leaves, as often prepared in Indonesia. Sometimes, pandan leaves impart green colour to the rice too.

In addition, some food industries produce pandan cake from the essence which is juices extracted from pandan leaves. Sometimes, pandan leaves are used as the fragrant wrappers for Thailand cuisine. For example, Pandanus chicken is known as *gai hor bai toey.*It is a classical recipe and an eternal favourite in some Thai restaurants. They use pandan leaves to wrap these marinated chicken bits and then deep-fry them in a wok. Besides, the most important culinary application of pandan leaves is discovered in desserts all over the South East. For instances, young coconuts iced drinks together with pandan flavour are popular in Thailand. Some pandan leaves are made into ice cream and sold in market (Katzer, G., 2001). Pandan leaves are also frequently added in sweet puddings or custards prepared from sticky, glutinous rice. For these concoctions, water, palm sugar, pandan leaves and glutinous rice are boiled together to produce a heavy mass rice. Then, it becomes semi-solid when cooling. The thick coconut milk normally is sprinkled over it before serving.

2.3 ESSENTIAL OILS

Essential oils are natural volatile and liquid aroma compounds extracted from the various plants. They actually are not oil but have the similar properties with oil which is poor solubility in water. They also exhibit the distinctive scent and flavor which depend on the oxygenated compounds. In general, essential oils consist of terpenoids, few are benzene derivative, but their compounds mainly depend on the type of solvent and extraction methods. Table 2.1 shows the major compound inside essential oils derived from some common plants.

Table 2.1: Essential oils from some common plants

Source: Mohammad, S., Bin I., (2008)

Name	Part of plant extracted	Botanical name	Important constituent	Usages
Lemongrass and citronella	Leaf	*Cymbopogonspp*	Citral, Citronella, Terpenes	Perfumery Disinfectant
Eucalyptus	Leaf	*Eucalyptus globules Eucalyptus citriodora Eucalyptus dives*	Cineale Citronella Terpenes	Not mention
Lavender	Flower	*Lavendulaintermedia*	Linalol	Perfumery
Clove	Bud	*Eugenia caryophyllus*	Eugenol	Dentistry flavouring
Sandalwood	Wood	*Santaium album*	Sanatols	Perfumery
Nutmeg	Nut	*Myristicafragrants*	Myristicin	Not mention
Almond	Nut	*Pruniscommunts*	Benzaldeyhde	Not mention
Coriander	Seed	*Coriandrumsatiyum*	LinalolTerpenes	Not mention
Garden Angelica	Seed	*Angelica archangelia*	β-terebangeleneTerpenes	Medical Flavoring
Oregano	Leaf	*Origanumvulgare*	ThymolCarvacol	Fungicide Digestive

Essential oils can be divided into two broad categories according their volume:

I. Large volume oils are usually extracted from the leafy material. (e.g. lemongrass, cinnamon leaves, oregano and citronella)

II. Small volume oils are extracted from the fruits, seeds, buds and lesser extent, flowers. (e.g. Clover, Lavender, Coriander and Almond)

Normally, pure essential oils are very expensive because it is produce from large quantity of plant material. However, few drops of essential oil are required to achieve the desired effect on the product. They have an intermediate impact to human's sense of smell known as olfaction. When we inhale the fragrance of essential oil, olfactory receptor cells are stimulated and the impulse is transmitted to the central of the brain known as limbic system. This limbic system is linked to areas of the brain which controls the memory, breathing and blood circulation. Furthermore, the endocrine glands which regulate hormone levels in the body will be affected by limbic system. Therefore, properties and fragrance of essential oils will affect the simulation of these systems in body.

Sometimes, these oils are used in the process of massage. Human not only inhale, also absorb them through skin. They penetrate the skin and flow into bloodstream inside our body. After that, they are transported to every organs and parts of our body. Some essential oils also will be absorbed quicker into skin via the hair follicles when applied to our body. The time for body absorbs the essential oils is depending on the concentration of essential oils. High quality pure essential oils are proved to have 70 times higher concentration than plant source. As a result, our brain and body will start to react to the vapor form of essential oils in less than four seconds after they stimulate the olfactory nerves and other sensors.

In the daily life, these essential oils may be used singly or in combination to to bring the curative and restorative effect to our mind and body. It is also a gentle alternative to modern hazardous drugs. They are applied already to be an assistance material in the treatment of the physical, mental and emotional changes, skin care and therapeutic massage. They can enhance and enrich positively our daily life even used solely for sensual pleasure.

2.4 PROPERTIES OF ESSENTIAL OIL

Essential oils mainly consists of hydrocarbons derivatives, so they exhibit the properties of hydrophobic and more soluble in organic solvents. However, they also can dissolve slightly in the water to give an intense odor to the solution such as rose water and orange flower water. The chemical compounds inside essential oils are volatile enough to distill unchanged in most methods, but they will decompose when the temperature is too high. Essential oils derived from different type of plant will have different colour. They vary from the colour to yellow or brownish in colour. They also have high refractive indexes which averaging about 1.5. Apart of that, these oils show wide range of optical activity and can rotate in both directions. The chemical compounds inside the essential oils can be classified based on their functional group as in next page:

Table 2.2: Functional groups of chemical compounds

Source: Mohammad, S., Bin I., (2008)

Functional groups	Common constituents
Carboxylic acid	Benzoic, Acetic, Salicyclic and Cinnamic acids
Alcohol	Linalool, Geraniol, Terpinol, Menthol, Borneol
Aldehydes	Citral, Benzaldehyde, Cinnamaldehyde, Vanilin
Phenols	Eugenol, Thymol, Carvacrol
Terpenes	Camphene, Pinene, Limonene, Phellandrene, Cedrene
Hydrocarbons	Cymene, Styrene
Ester	Benzyl Acetate, Triglycerides, Phospholipids

In general, these constituents are mainly built from five species of atoms which are carbon, hydrogen, oxygen, nitrogen and sulphur. Nitrogen and sulphur have much lesser extent inside the essential oils. Nowadays, new analytical techniques have been created to study the chemical compounds inside these oils. As a result, the number of